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Cytokines and the Brain 2

A Satellite Symposium to the Society for Neuroscience 30th Annual Meeting

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Programme

Thursday, November 2, 2000

Cytokines in Ischemia: Neurotoxic or Neuroprotective?

Chair: Patricia E. Molina, M.D., Ph.D.

Patricia E. Molina Louisiana State Univ Health Sciences Ctr. New Orleans, LA. USA	Overview and Introduction
Giora Z. Feuerstein DuPont. Wilmington. DE. USA	The Janus face of inflammation in brain injury
Esther Shohami The Hebrew Univ School of Pharmacy. Jerusalem. Israel	Dual role of TNFα in brain injury: early detrimental effect is enhanced by reactive oxygen species
Sarah A. Loddick University of Manchester. UK	Interleukin-1 and interleukin-6 – opposing actions in the ischaemic brain?
Kevin J. Tracey North Shore University Hospital. New York Univ School of Medicine. Manhasset. NY. USA	TNF. brain ischemia and the vagus nerve
Felix P. Eckenstein Oregon Health Sciences Univ. Portland. OR. USA	Are chemokines the key for understanding genetic differences in neuronal vulnerability?

Cytokines and Neurodegenerative Disease

Chairs: Douglas E. Brenneman, Ph.D. and Akira Arimura, M.D., Ph.D.

Illana Gozes Tel-Aviv Univ. Tel-Aviv. Israel	Femtomolar peptide-based neuroprotection in vivo: gene array assessments of cytokine mRNA expression
Jan Hong NIEHS. Research Triangle Park. NC. USA	Roles of microglial activation in inflammation-related neurotoxicity: Neuroprotective effects of opioids
Mark P. Mattson NIA. Baltimore. MD. USA	Cytokines and survival of CNS neurons: Focus on NFκB and ceramide
Keith W. Kelley Univ of Illinois. Urbana. Illinois. USA	Silencing of a neuronal survival signal by tumor necrosis factor α
Douglas E. Brenneman. NICHHD. Bethesda. MD. USA	Cytokine release from astrocyte cultures: Comparison between normal and segmental trisomy mouse brain
Robert M. Friedlander Brigham and Women's Hospital. Harvard Medical School. Boston. MA. USA	Role of caspases in neurological disease.

Poster Session

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Friday, November 3, 2000

Cytokine Interactions with Neurotransmitters, Neuropeptides, Growth Factors, and Hormones

Chairs: Adrian J. Dunn, Ph.D. and Akira Arimura, M.D., Ph.D.

Adrian J. Dunn Louisiana State Univ Health Sciences Ctr, Shreveport, LA, USA	Overview of cytokine effects on brain neurotransmission
Akira Arimura Tulane Univ, Belle Chase, LA, USA	Cytokine interactions with urocortin during stress
Mario Delgado Complutense Univ, Madrid, Spain	Macrophages and microglia as targets for the anti-inflammatory role of VIP and PACAP
Ljubisa Vitkovic Bethesda, MD, USA	Mechanism of TGF-β1 secretion in glial cells

Mechanisms for Cytokine Signalling

Chairs: Clark M. Blatteis, Ph.D. and Steven F. Maier, Ph.D.

Lisa Goehler Univ of Virginia, Charlottesville, VA, USA	Immunosensory signal transduction by the vagus: role of cytokines
Andrej A. Romanovsky St. Joseph's Hospital and Medical Center, Phoenix, AR, USA	Neuroimmunomodulation of fever: The multiple roles of the vagus
Giamal N. Luheshi Douglas Hospital Research Center, McGill University, Quebec, Canada	Blood-borne cytokine signaling mechanisms: the role of circulating IL-6 in fever
Kiyoshi Matsumura and Shigeo Kobayashi Univ of Kyoto, Kyoto, Japan	Brain endothelial cells are the primary sites for cytokine action and prostaglandin synthesis
Clark M. Blatteis Univ of Tennessee Health Science Center, Memphis, TN, USA	Fever induction by endotoxin: Are cytokines, prostaglandins or other molecules the primary signals?

Saturday, November 4, 2000

Cytokines and Behavior: Effects on Learning, Memory, Depression, Sleep, and Appetite

Chairs: Steven F. Maier, Ph.D. and Adrian J. Dunn, Ph.D.

Zul Merali Univ of Ottawa, Ottawa, Canada	Differential time-dependence sensitization effects elicited by tumor necrosis factor-α: HPA activation, sickness behavior and brain monoamine activity
Marina Lynch Trinity College, Dublin, Ireland	Analysis of the mechanism underlying the inhibitory effect of IL-1β on LTP in rat dentate gyrus
Steven F. Maier Univ of Colorado, Boulder, CO, USA	Brain interleukin-1 and memory formation
James M. Krueger, Washington State Univ, Pullman, WA, USA	Cytokines in physiological sleep regulation
Raz Yirmiya The Hebrew Univ of Jerusalem, Israel	Cytokine-mediated emotional and cognitive disturbances in rodents and humans
Robert Dantzer INSERM, Bordeaux, France	What is the evidence for a role of cytokines in depression?
Arnaud de Beaurepaire Hopital Paul Guiraud, Villejuif, France	Discussant

Cytokines in Ischemia: Neurotoxic or Neuroprotective?

Brain Cytokines and Ischemia; Neuroprotective or Neurotoxic Effects?

Molina P.E.

Department of Physiology, LSUHSC, New Orleans, LA, USA

Over the past few years, a body of evidence has stressed the role of inflammation in the pathophysiology of acute ischemia. Studies have demonstrated that the majority of the inflammatory reactions that are triggered by an ischemic insult are mediated by cytokines. Mild to moderate brain ischemia, resulting from hemorrhagic shock has been shown to be sufficient to up-regulate tissue (including brain) pro-inflammatory cytokine levels in animal experiments. This ischemic-induced up-regulation of tissue cytokines is closely regulated by neural and opiate mechanisms. The localized release of pro-inflammatory cytokines results in up-regulation of adhesion molecules, recruitment and activation of neutrophils leading to reactive oxygen species (ROS) release. The rise in tissue pro-inflammatory cytokines (IL-1, TNF-α, and IL-6) has been reported to occur early on in the ischemic cortex in experimental models of stroke. Furthermore, results from some human studies suggest that tissue cytokine expression exhibit differential pattern between glial cells and neurons, and that the magnitude of the cytokine response relates to the extent of infarcted area. These cytokines are hypothesized to be interactive as indicated by the difference in time course of their expressions, with IL-1α being the earliest and IL-6 being the latest. Although not well defined, their source could include glial cells, neurons or infiltrating circulating monocytes. Recent studies indicate that in addition to their central role in the inflammatory response, cytokines are also involved in nerve regeneration; thus potentially having pleiotropic effects dependent on localization, prevailing neurochemical milieu and time-course. The interplay between glial cells, infiltrating leukocytes and induced cytokines in the immediate inflammatory process as it pertains to regulation of excitotoxicity, synergism with ROS and tissue injury is incompletely understood. This session will focus on recent studies aimed at understanding the role of increased expression of tissue cytokines during brain ischemia.

The Janus Face of Inflammation and CNS Injury

Feuerstein G.Z.

DuPont Pharmaceuticals Company, Experimental Station, Wilmington, DE, USA

Ischemic or traumatic injury to the brain results in numerous biochemical and molecular perturbations. In recent years, evidence has been raised to suggest that inflammation is a major component of the brain response to ischemia or trauma. The evidence in support for this position is derived from studies that demonstrate infiltration of inflammatory cells from the circulation into the injury zone and also the activation of endogenous inflammatory cells-microglia in response to injury. Furthermore, shortly after injury, brain capillaries express adhesion molecules such as E/P-selectins and ICAM-1, which mediate leukocyte-endothelium interaction. Furthermore, cytokines (TNFα, IL-1β, IL-6) and chemokines (MCP-1, IP-10, IL-8) that induce chemoattraction for inflammatory cells and are induced de novo by both neurons and glia cells and neurons. Evidence in support for a potential detrimental role for inflammatory cells and mediators has been derived from studies where depletion of neutrophils resulted in lesser injury following ischemic injury and that antagonists to TNFα or IL-1β (e.g., IL-1ra) ameliorated the histological and functional consequences to this injury. However, studies conducted with cytokines such as TNFα applied before ischemic injury demonstrated some tolerance of the brain tissue to ischemia. Furthermore, studies conducted on genetic models of TNFα or TNFα receptor deficiency mice have shown clear exacerbation of functional and histological outcome following an ischemic or traumatic injury. Taken together, the mounting evidence at this time suggests that the role of inflammation in brain injury may be dual: some mediators acting acutely may contribute to tissue and functional damage while the inflammatory reaction at large, on the long term, may be important in repair and recovery from ischemic or traumatic injuries.

Dual Role of Tumor Necrosis Factor α in Brain Injury: Early Detrimental Effect Is Enhanced by Reactive Oxygen Species

Shohami E.[a], *Ginis I.*[b], *Trembovler V.*[a], *Spatz M.*[b] and *Hallenbeck J.M.*[b]

[a] Department of Pharmacology, The Hebrew University of Pharmacy, Jerusalem, Israel and [b] Stroke Branch, Institute of Neurological Disorders and Stroke, [illegible], Bethesda, Maryland, USA

There is conflicting evidence on the role of TNFα in brain: Whereas ample evidence show that pharmacological inhibition of TNFα after ischemia or trauma is beneficial, TNFα knock-out mice suggest the opposite. Moreover, studies demonstrate that TNFα may exhibit neuroprotec-

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crepancy suggests that other pathogenic stimuli induced in the settings of brain injury may precipitate TNFα cytotoxicity. In an animal model of closed head injury we showed that exogenous and endogenous antioxidants were cerebroprotective even when TNFα levels were high, and hypothesized that reactive oxygen species (ROS) released early after injury alter TNFα signaling pathways to cause cell death. This hypothesis was tested in PC12 cells, in microvascular endothelial cells and in cortical astrocytes. For each cell type we selected doses of TNFα and H_2O_2 that individually were not toxic to the cultures, and added them simultaneously (for 18–24 h). Markers of cellular stress response, death and apoptosis were quantified, and activation of the transcription factor NF-κ B was studied. In PC12 cells, LDH release and PGE_2 production served as respective markers for cellular damage and induced stress-response. Under concentrations where neither TNFα nor H_2O_2 alone had any effect, the combined effect was marked, and massive release of LDH was accompanied by accumulation of PGE_2. Similarly, in astrocytes and endothelial cells, TNFα and H_2O_2 synergize to cause drastic cell death (quantified by ethidium exclusion test) and apoptosis (TUNEL staining). Western blot analysis of TNFα-induced activation of NF-κB demonstrated that sublethal doses of H_2O_2 inhibited translocation of the p65 subunit of NF-κB to the nucleus, with no effect on I-κB degradation in cytoplasm. Immunostaining with the antibody directed against p65 demonstrated that H_2O_2 inhibited p65 transport to the nucleus. Thus, by creating a neurochemical milieu that simulates the injured brain we showed that the simultaneous presence of high levels of ROS and TNFα is detrimental.

Interleukin-1 and Interleukin-6 – Opposing Actions in the Ischaemic Brain?

Loddick S.A.

Biological Sciences, University of Manchester, Manchester, UK

The pro-inflammatory cytokine interleukin-1 (IL-1) has been implicated in the development of ischaemic brain damage. Injection of rIL-1β into the brain of rats subjected to ischaemia (by middle cerebral artery occlusion, MCAO) results in a dramatic increase in brain injury. Conversely, injection of the recombinant form of IL-1 receptor antagonist (IL-1ra) results in a dramatic reduction of ischaemic brain injury, implicating endogenous IL-1 in the development of damage. Interleukin-6 (IL-6) is a related cytokine that shares many actions with IL-1 in the periphery. Chronic overexpression of IL-6 in the brain results in profound neurology, indicative of a damaging effect of IL-6. To investigate the role of IL-6 in acute ischaemic brain injury we first measured IL-6 bioactivity in the brain of rats subjected to ischaemia (MCAO). Ischaemia caused a dramatic increase of IL-6 bioactivity in the ischaemic hemisphere as early as 2 h after MCAO. To investigate the potential role of IL-6 in the ischaemia we injected rIL-6 into the brain of ischaemic rats using doses similar to those we detected previously in the ischaemic hemisphere. Both doses of rIL-6 caused a significant reduction of the lesion volume, indicating protective effects of IL-6 in acute ischaemic brain damage. These data indicate complex roles of cytokines in ischaemic brain damage, further investigations are needed to elucidate the relationship between IL-1 and IL-6 in these circumstances.

TNF, Brain Ischemia and the Vagus Nerve

Tracey K.J.

Laboratory of Biomedical Science, North Shore University Hospital, New York University, NY, USA

The brain and immune systems detect and remember the identity of threatening stimuli. Mediators produced by macrophages and other immunocompetent cells activate CNS responses; output from the CNS in turn modulates the immune response. TNF is a proinflammatory cytokine that occupies a critical early role integrating the host response to injury, infection, and ischemia. For instance, during cerebral ischemia, neurons and astrocytes in the ischemic penumbra express high levels of TNF that mediates the progression of cell death (Molecular Medicine 1997, 3:765–81). Brain damage is significantly reduced with experimental therapeutics that inhibit either TNF activity (e.g. anti-TNF antibodies), or TNF expression (e.g., CNI-1493, a tetravalent guanylhydrazone inhibitor of TNF synthesis) (Shock 1997, 8:341–8). While investigating the phenomenon associated with intracerebral administration of CNI-1493 to inhibit TNF, we realized that intracerebroventricular application of CNI-1493 also significantly suppressed the peripheral TNF response to endotoxin. Effective blockade of serum TNF was achieved by applying CNI-1493 into the cerebral ventricles in doses 30,000-fold less than required to suppress TNF after intravenous dosing. Surprisingly, the TNF suppressing signal from the brain to the periphery was carried in the vagus nerve. CNI-1493 significantly increased activity in the efferent vagus nerve, and vagotomy abolished the inhibition of TNF by intracerebral CNI-1493 (J. Autonomic NS, in press). Direct electrical stimulation of the efferent vagus nerve in the absence of CNI-1493 significantly inhibited TNF synthesis in liver, suppressed peak serum TNF levels, and conferred significant protection against the development of endotoxin-induced shock (Nature 2000, 405:458–62). Acetylcholine released by the vagus nerve interacts with nicotinic, alpha-bungarotoxin sensitive receptors on macrophages that transduce signals to suppress proinflammatory cytokine release through post-transcriptional mechanisms (Nature 2000, 405:458–62). Thus, from studies originally focused on convergence of cytokine activities in cerebral ischemia came the discovery of a vagus nerve-CNS mechanism that inhibits peripheral immune responses, now termed the 'cholinergic anti-inflammatory pathway.'

Are Chemokines the Key for Understanding Genetic Differences in Neuronal Vulnerability?

Eckenstein F., Kulhanek D. and Woodward W.R.

Departments of Cell and Developmental Biology and Neurology, Oregon Health Science University, Portland, OR, USA

Excitotoxic neuronal cell death is thought to contribute to the damage caused by ischemia. The present study addresses whether inflammatory mechanisms may contribute to delayed hippocampal pyramidal cell death observed after peripheral injection of the neurotoxin, kainate. Interestingly, genetic differences between mouse strains make some strains (e.g. FVB/N) more vulnerable to kainate than others (e.g. C57Bl6: see Schauwecker et al *PNAS* 94: 4103–

4108, 1997). We investigated the regulation of expression of over 30 cytokine and chemokine mRNAs by Multiprobe Ribonuclease Protection Assay (MRPA) and the overall regulation of gene expression by DNA microarray technique in this animal model. The MRPA method revealed a striking induction of several chemokine mRNAs 3 hours after kainate treatment, which was sustained for up to 72 h. This induction was seen only in FVB, but not in C57 mice. Unexpectedly, pro-inflammatory cytokine mRNAs were not markedly induced in the same samples. Microarray results showed that for the most part gene expression is similar between untreated FVB and C57 mice, but that mRNAs coding for midkine and fibroblast growth factor receptor 3 (FGFR3), both molecules with potential neurotrophic function, are expressed at significantly higher levels in C57 than in FVB mice. At 3 h after kainate injection, genes encoding mediators of intracellular signaling pathways and cell cycle regulators are among those most highly upregulated, and there are more changes in gene expression in FVB mice than in C57 mice. At 24 h after kainate injection, the expression of an even larger number of genes are changed, and there is only a partial overlap of the genes whose expression is changed at 3 and 24 h. Together these data suggest the hypothesis the action of chemokines may contribute to hippocampal neuronal vulnerability to excitotoxicity and that C57 mice may be less sensitive to kainate injury due to higher levels of expression of neurotrophic agents.

Cytokines and Neurodegenerative Disease

Gene Array Assessments of Cytokine mRNA Expression

Gozes I.[a], *Romano J.*[a], *Beni-Adani L.*[b], *Steingart R.A.*[a], *Levy Nissenbaum O.*[a], *Brenneman D.E.*[d] *and Shohami E.*[c]

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Activity-dependent neuroprotective protein (ADNP, J. Neurochem. 72: 1283–1293 [1999]; J. Biol. Chem., in press) contains a femtomolar-acting eight-amino-acid peptide (NAP) that provided long-term protection against memory deficits in rats suffering from cholinotoxicity (J. Pharmacol. Exp. Therap. 293: 1091–1098 [2000]). Recent data suggested that a single injection of NAP, protected against the deleterious outcome of closed head injury (CHI) in mice (J. Pharmacol. Exp. Therap., in press). Part of the mechanism leading to neuroprotection in CHI entailed inhibition of immediate increases in TNFα production and protection against TNFα toxicity. Here, 30–45 days after CHI, mice that exhibited similar clinical outcome were sacrificed and total cerebral cortex RNA was prepared. Resulting radioactive cDNA preparations were hybridized to Atlas Array membranes containing 1200 cDNAs spots (Clontech, Palo Alto, CA, USA). One of the genes exhibiting an interesting pattern of expression was the interleukin 6 (IL-6) signaling transducer glycoprotein 130 (gp 130), implicating the IL-6 signaling pathway in the long-term outcome of head injury and NAP mechanism of action.

Supported by ISF, BSF, ISOA, Lily and Avraham Gildor Chair for the Investigation of Growth Factors.

Roles of Microglial Activation in Inflammation-Related Neurotoxicity: Neuroprotective Effects of Opioids

Hong J.S. and Liu B.

Laboratory of Pharmacology and Chemistry, NIEHS/NIH, Research Triangle Park, NC, USA

The major goal of this project was to examine the role of opioid systems in modulating the expression of inflammatory [illegible] in microglia and in the mediation of immune-related neurodegeneration. We have employed both in vitro and in vivo rodent models of Parkinson's disease by focusing our studies on the mesencephalic dopaminergic neurons. Our laboratory was among the first to [illegible] that lipopolysaccharide (LPS)-induced neurotoxicity depends on the presence of glial cells. These cells secrete a variety of pro-inflammatory factors, including cytokines, free radicals and arachidonic

metabolites that are the main contributors to the pathogenesis of inflammation-related neurodegenerative diseases. Our current efforts focus on determining the relative importance of these proinflammatory factors in glia-mediated neuronal damage. We have examined the effects of opioids on the activity of microglia and its relationship to inflammation-related neurodegenerative diseases. Among the different families of opioid peptides studied, the most important finding was the potent neuroprotective effects of ultralow concentrations (10^{-14}–10^{-16} *M*) of dynorphins against LPS-induced damage to dopaminergic neurons. We observed these effects in both mixed mesencephalic neurons/glia cultures and animal models. Immunocytochemical analysis revealed that the reduction of LPS-induced activation of microglia by dynorphins was associated with their neuroprotective effects. Another intriguing finding from this series of studies was the observation that naloxone, an opioid receptor antagonist, exhibited the same neuroprotective efficacy as dynorphins, which are opioid receptor agonists. Pharmacological studies using different analogs of dynorphins and optical isomers of naloxone demonstrated that the inhibitory effects on microglial activity and the neuroprotective effect of both dynorphins and naloxone were not mediated through the classical opioid receptors. These results explained why both opioid agonists and antagonists exert similar effects. Moreover, our studies revealed important non-opioid actions of these two opioid compounds. Further studies are planned to elucidate the mechanism for both dynorphin- and naloxone-mediated inhibition of microglia activation and their mode of neuroprotective actions. Our studies should provide a new strategy in the search for novel neuroprotective agents of clinical benefit.

Cytokines and Survival of CNS Neurons: Focus on NFκB and Ceramide

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Cytokine cascades are activated in the brain in response to cellular stress associated with physical and ischemic insults and in chronic neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. These cascades, which involve tumor necrosis factor, interleukins and interferon-γ may have either beneficial or detrimental effects on neuron survival. Activation of the transcription factor NF-κB in neurons increases their resistance to apoptosis induced by excitotoxic, oxidative and metabolic insults, whereas activation of NF-κB in microglia may enhance their ability to kill neurons (for review see J. Neurochem. 74: 443–456 (2000)). We have recently identified novel mechanisms for activation of NF-κB (e.g., release of a signal from endoplasmic reticulum in response to depletion of IP3-sensitive stores) and for neuroprotection by NF-κB (e.g., modulation of expression of antioxidant enzymes, Bcl-2 family members and glutamate receptor subunits). An important injury-responsive signaling pathway involves cleavage of membrane sphingomyelin by acidic sphingomyelinase (ASMase) resulting in generation of the second messenger ceramide. Our recent studies in a stroke model (Yu et al., J. Mol. Neurosci. In press) show that transient focal cerebral ischemia induces large increases in ASMase activity, ceramide levels and production of inflammatory cytokines in wild-type mice, but not in mice lacking ASMase. The extent of brain tissue damage is decreased and behavioral outcome improved in mice lacking ASMase. Neurons lacking ASMase exhibit decreased vulnerability to excitotoxicity and hypoxia which is associated with decreased levels of intracellular calcium and oxyradicals. Treatment of mice with a drug that inhibits ASMase activity and ceramide production reduces ischemic neuronal injury and improves behavioral outcome. Collectively, our data suggest that drugs that modulate ceramide and NF-κB signaling pathways may prove beneficial in an array of neurodegenerative conditions.

Silencing of a Neuronal Survival Signal by Tumor Necrosis Factor α

Kelley K.W.[a], *Venters H.D.*[a], *Bluthé R.-M.*[b] *and Dantzer R.*[b]

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Tumor necrosis factor-α (TNFα) is well known to be involved in the pathology of type II diabetes by inducing resistance of the insulin receptor. We recently demonstrated that very low concentrations of TNFα induce a similar resistance phenomenon following activation of the closely related receptor for insulin-like growth factor (IGF-1) on primary murine cerebellar granule neurons (CGN; Proc. Natl. Acad. Sci. USA, 1999, 96:9879). IGF-1 potently increases survival of CGN by ~ 6-fold. These cells express receptors for both the p55 and p75 isoforms of the TNF receptor. At concentrations of 1 ng/ml or less, TNFα exhibits neither cytotoxic nor protective activity. However, pretreatment of CGN with as little as 10 pg/ml TNFα reduces by 50% the ability of IGF-1 (100 ng/ml) to protect against neuronal cell death. To explore the molecular mechanism for this phenomenon, we found that CGN express only one of the two major docking molecules utilized by the IGF-1 receptor, insulin receptor substrate-2 (IRS-2). Tyrosine phosphorylation of this 185-kDa docking molecule is increased 5-fold by IGF-I. Preincubation with TNFα (10 pg/ml) reduces the ability of IGF-1 to tyrosine phosphorylate IRS-2 to 1.6 fold. The p85 regulatory subunit of phosphatidylinositol 3′ kinase (PI 3-kinase) binds to IRS-2 via its two Src homology 2 domains (SH2), and this leads to activation of the p110 catalytic domain of PI 3-kinase. In CGN, IGF-1, but not TNFα, causes a dose-dependent increase in activity of PI 3-kinase, which is essential for the survival-promoting activity of IGF-1. However, when CGN are cultured with both IGF-1 (100 ng/ml) and TNFα (10 pg/ml), activity of this critical survival enzyme is significantly reduced one half. We have termed this new concept in neurodegeneration 'Silencing of Survival Signals' (SOSS; Trends in Neurosciences, 2000, 23:175) because it points to a different mechanism by which TNFα regulates the life and death of CGN. This model might also explain why TNFα has been reported to both inhibit and promote neuronal survival. In the relative absence of other survival factors, selective concentrations of TNFα may modestly promote survival of neurons. However, in the presence of abundant survival factors such as IGF-1, TNFα potently leads to neuronal death, even at picogram concentrations. These results show that

TNFα promotes the death of CGN by silencing IGF-1 survival signals.

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Cytokine Release from Astrocyte Cultures: Comparison between Normal and Segmental Trisomy Mouse Brain

Brenneman D.E.[a], *Hauser J.*[a], *Mokolla M.*[a], *Hill J.M.*[a], *Ring M.*[a], *McCune S.K.*[b], *Ades A.M.*[b], *Crnic L.S.*[c] and *Phillips T.M.*

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Segmental trisomy (Ts65Dn) mice have been used as a model of neurodegeneration and memory dysfunction. Characteristics of the Ts65Dn mouse include loss of choline acetyl transferase immunoreactivity in the basal forebrain, decreases in cortical synapses and impaired performance on leaning and memory paradigms. In the present study, astrocyte cultures were prepared from the cerebral cortex of aged (12–15 months) Ts65Dn mice and age-matched controls. Spontaneous and VIP-mediated release of cytokines were measured after one hour incubations. The spontaneous release of TNF-α, IL-1α, and MIP-1α was 6–15-fold greater in the conditioned medium from Ts65Dn-derived astrocytes in comparison to that of controls. In contrast, the release of IL-6 was not significantly different between normal and Ts65Dn cultures. Treatment with the neuroprotective peptide VIP inhibited the release of the cytokines from both Ts65Dn and control cultures. These studies indicate that astrocytes derived from aged Ts65Dn mice secrete significantly greater amounts of cytokines in comparison to controls and these changes could contribute to the cholinergic deficits and memory impairment observed in this model. These studies further demonstrate a regulatory role for VIP on cytokine secretion from astrocytes that may account for it's neuroprotective and neurotrophic functions.

Role of Caspases in Neurological Disease

Friedlander R.M.

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The functional role of the caspase cell death family in neurodegeneration, in particular ALS, has been clearly demonstrated. We have shown that caspases-1 and -3 are regulated at the transcription level in the mutant superoxide dismutase $(SOD)1^{G93A}$ transgenic ALS mouse model. Caspases-1 and -3 are specifically activated in ventral horn neurons in this mouse model. Adding relevancy to this finding, caspase-1 and -3 activation have been demonstrated in spinal cord of humans with ALS. Caspase inhibition, either by the caspase-1 dominant negative transgene, or by administration of the broad caspase inhibitor zVAD-fmk, slows disease progression and delays mortality in mutant $(SOD)1^{G93A}$ mice. Elevated levels of mature IL-1β are detected in the spinal cord of humans and mice with ALS. Furthermore, inhibition of mature IL-1β production correlates with inhibition of cell death as well as delayed mortality. A proper knowledge of the caspase-mediated pathways will aid in designing rational pharmacotherapy for ALS. Since the mechanisms of cell death in these devastating diseases appear to be shared, furthering the understanding of the mechanisms of neurodegeneration in ALS will likely result in benefits to other neurodegenerative diseases such as Huntington's, Parkinson's, and Alzheimer's disease.

Cytokine Interactions with Neurotransmitters, Neuropeptides, Growth Factors, and Hormones

Cytokine Effects on Cerebral Neurotransmission

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It has been twelve years since the discovery that peripheral administration of interleukin-1 (IL-1) to mice and rats increased the metabolism of norepinephrine (NE) and serotonin (5-HT). The effects of IL-1 on NE metabolism were most marked in the hypothalamus, but were observed in all regions of the brain examined. This response was quite slow, peaking at around 2 h, and dissipating around 4 h. IL-1 also increased brain tryptophan (Trp) in a regionally nonspecific manner, that did not appear to be related to the presence of serotonergic neurons. Increases in 5-HIAA, the major catabolite of 5-HT, closely paralleled those of Trp. These responses were considerably slower than those in NE, peaking at around 4 h and lasting about 8 h. It is now known that other cytokines can also affect cerebral neurotransmitter metabolism, but none yet studied is as potent as IL-1. Tumor necrosis factor α (TNFα) has been shown to increase the metabolism of NE and to increase Trp. Interleukin-6 has no effect on NE, but increases Trp and 5-HIAA just as does IL-1. IL-2 has complex effects on dopamine metabolism, but interferon α had no detectable effect on these transmitters. There are scattered reports of effects of IL-1 on acetylcholine, histamine, as well as the amino acid neurotransmitters, glutamate, glutamine and GABA. The effects of IL-1 on NE and 5-HT appear to reflect increased synaptic release of these neurotransmitters, because microdialysis studies show increased extracellular concentrations. This is also true for the effect of IL-6 on 5-HT. Despite the clear demonstrations of effects of cytokines on brain neurotransmitters, the functions are unknown. Some evidence supports a role for the noradrenergic response in the hypothalamopituitary-adrenocortical (HPA) activation caused by IL-1, although NE does not appear to be essential for this activation. Although it seems likely that the noradrenergic and serotonergic responses underlie some of the behavioral changes observed following cytokine administration (e.g., sickness behavior), experiments to test this have failed to provide supporting evidence.

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Urocortin and Cytokines during Stress

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Corticotropin-releasing factor (CRF) plays a key role in the acute self-defense response to stress by stimulating the release of ACTH and increasing sympathetic tone through brainstem pathways. In addition, CRF is expressed in peripheral tissues. We previously demonstrated that immobilization (IM) stress resulted in an increase in plasma IL-6 levels and that this response was suppressed by pretreatment with a CRF receptor antagonist or injection of a CRF antiserum. This suggests that tissue CRF modulates stress-induced IL-6 expression. However, because the expression of CRF in the peripheral tissues is very limited, it is difficult to consider that tissue CRF has a significant modulatory effect on the expression of cytokines during stress. The recently identified CRF-like peptide urocortin (UCN) was found to bind to CRFR-2 receptors which are expressed both in the brain and the peripheral tissues. By activating CRFR-2 receptors, UCN could affect autonomic functions and modulate the efferent components of the endocrine, immune and behavioral responses to stress. An intraperitoneal injection of UCN increased circulating levels of IL-6 to a greater extent than CRF. In the brain, UCN immunostained neurons are most abundant in the Edinger-Westphal nucleus (EW), though the physiological role of UCN is unknown. Double-labeling immunohistochemistry (IHC) showed that an acute pain stress increased Fos expression as well as the number of UCN-positive neurons in EW. Fos was nearly exclusively expressed in UCN-positive neurons in EW. The levels of UCN mRNA in the EW also increased. On the other hand, neither Fos nor UCN expression in EW changed after hemorrhagic stress, suggesting that these UCN-containing neurons respond preferentially to sensory stimuli. In peripheral tissues, a considerable amount of immunoreactive UCN was found in the stomach, spleen, adrenal glands, and thymus. IHC showed that nearly all parietal cells of the stomach were UCN-positive and these cells also expressed tyrosine hydroxylase. One hour after hemorrhage, plasma UCN levels increased, remained elevated for about 2 h after resuscitation and then decreased to the pre-hemorrhage levels. However, in animals that died after hemorrhage, UCN levels remained high or rose further. In general, plasma UCN levels showed a sluggish rise after hemorrhage. In splenocyte cultures, addition of UCN ($< 10^{-8}$ *M*) alone did not alter IL-6 production, but augmented IL-1-stimulated IL-6 production, as does norepinephrine (10^{-8} *M*). However, although its synergistic effect was significant, the extent was moderate and cannot be considered to be a critical modulatory effect on IL-6 production. On the other hand, UCN appears to play a significant inhibitory role in TNFα expression in various organs following hemorrhage. The findings suggest that UCN, both in the brain and the peripheral tissues, shows a subacute or sluggish response to stress, and regulates the stress-induced cytokine response, but its effect on the expression of IL-6 and TNFα was in opposite directions.

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Macrophages and Microglia as Targets for the Anti-Inflammatory Role of VIP and PACAP

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Inflammatory processes contribute to neurodegenerative disease, stroke, encephalitis, and other central nervous system (CNS) disor-

ders. In response to brain injury, brain microglia become activated, in a way similar to peripheral tissue macrophages, a process which includes differentiation and probably invasion and proliferation. Activated microglia are a source of cytokines and other inflammatory agents within the CNS and it is therefore important to control glial function in order to preserve neural cells. Vasoactive intestinal peptide (VIP), and the structurally related pituitary adenylate cyclase-activating peptide (PACAP) are two neuropeptides produced by both nervous and immune system. Although both neuropeptides exert a broad spectrum of biological effects, they have been identified as two potent anti-inflammatory factors. VIP and PACAP show a protective effect against several inflammatory diseases such as endotoxic shock and arthritis. The systemic anti-inflammatory effect of both neuropeptides is exerted through the down-regulation of several macrophage-derived proinflammatory factors such as TNFα, IL-6, IL-12, IFNγ, nitric oxide, IL-18, and some chemokines (MCP-1, MIP-1α, MIP-1α, RANTES, MIP-2) and the upregulation of the anti-inflammatory cytokines IL-10 and IL-1ra. In a similar way than macrophages, VIP and PACAP affect endotoxin-induced production of both anti- and pro-inflammatory factors by microglia. The effect seems to be mainly mediated through VPAC1 receptor and the subsequent activation of cAMP/PKA pathway. This broad anti-inflammatory effect could be attributed to the ability of both neuropeptides to regulate several transduction pathways and transcription factors, including Jak-STAT and MEKK1-JNK pathways, and NFκB, AP-1 and IRF-1 factors. Because VIP and PACAP inhibit production of pro-inflammatory mediators by activated microglia they might be useful in treatment of inflammatory/degenerative brain disorders.

Mechanism of TGF-β1 Secretion in Glial Cells

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TGF-β1 is directly and/or indirectly, neurite-promoting, neurotrophic and neuroprotective in several diseases/disorders of the brain. It is synthesized as a large precursor and processed into biologically active, smaller protein. The processing is coupled to secretion and depends on both, its primary structure and complexing with other proteins that keep TGF-β1 inactive. Cultured glial cells constitutively secrete TGF-β1 and phago- or endocytosis stimulates the secretion probably via coupling of these processes in lysosomes. Chloroquine, an alkalizing agent, inhibits dose-dependently secretion of TGF-β1 but not protease nexin 1 (PN-1, another neuroprotective protein), and this inhibition occurs in cells with unaffected as well as elevated $[Ca^{2+}]i$. Brefeldin A, an inhibitor of secretory vesicle formation, reversibly inhibits release of both proteins. Mastoparan, a peptide that preferentially stimulates Go/Gi protein activity, elevates secretion of PN-1 but little TGF-β1. In cells overexpressing Goα, TGF-β1 secretion rate is also not affected, whereas PN-1 secretion is elevated. Taken together, these results suggest that TGF-β1 is secreted by a distinct constitutive secretory pathway that is coupled to acidic compartments and independent of Goα control. This may be relevant to stimulating TGF-β1 secretion in diseased brain.

Mechanisms for Cytokine Signaling

Immunosensory Signaling by the Vagus Nerve: Role of Cytokines

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Effective host defense requires the existence of a sensory system capable of detecting a wide range of pathogens, then signaling the nervous system to initiate body-wide defensive responses. In mammals this sensory system operates via both humoral and neural pathways. As a principal neural pathway, the vagus nerve is strongly implicated in the conveyance of immunosensory information deriving from internal tissues. As such, the vagus nerve can be used as a model to elucidate organizing features of immunosensory systems in general. For instance, how is immunosensory signal transduction accomplished? The vagus contains large populations of sentinel-type immune cells, which express cytokines including interleukin-1 rapidly upon treatment with immune stimulants. Sensory signal transduction likely occurs via specialized cytokine-receptor expressing glomus cells or via primary sensory neurons themselves. Among important targets for immunosensitive vagal neurons are lymph nodes, which represent a critical interface between pathogens and immune cells. Immune cells in lymph nodes express a variety of cytokines; however the role of these lymph node derived cytokines in immunosensory signaling is not well studied. Subtle differences are evident in the pattern of central nervous system activation by different immune stimulants. These patterns may derive from primary stimulus characteristics coded by cytokines.

Neuroimmunomodulation of Fever: The Multiple Roles of the Vagus

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The effects of vagotomy on the thermoregulatory response to systemic inflammation are reviewed, primarily for the model of intravenous (IV) lipopolysaccharide (LPS) administration in the rat. The following conclusions are drawn. 1) Vagotomy-associated thermoeffector insufficiency is likely to account for the attenuation of the fever response observed in some, but not all, studies; such an insufficiency is, however, preventable by postoperative care. 2) The febrile response to low doses of LPS (monophasic fever) is mediated by the hepatic (but not gastric or celiac) vagal fibers, presumably afferent; the same fibers are likely to be involved in the development of tolerance to low doses of circulating LPS. 3) Phase 1 of the polyphasic febrile response to moderate doses of LPS involves capsaicin-sensitive afferents (either nonvagal only or both nonvagal and vagal), does not involve cholecystokinin A-receptors, and may involve peripheral prostaglandins. 4) Febrile phase 2 does not require the integrity of abdominal nerve fibers, either vagal or nonvagal. 5) Phase 3 and

febrile response to IV LPS (and perhaps the response to intraperitoneal LPS) involves capsaicin-insensitive vagal fibers, presumably efferent. 6) A hepatoceliac vagal, presumably efferent, anti-inflammatory mechanism counteracts the development of LPS-induced hypothermia and shock.

Blood Borne Cytokine Signaling Mechanisms: The Role of Circulating IL-6 in Fever

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The nature of the peripheral signal to induce brain mediated host defense response to disease has been a subject of much controversy and continues to generate a great deal of debate. There is now overwhelming evidence supporting the hypothesis that neural afferents in the shape of the vagus nerve are the major route of relaying peripheral signals to the brain. These observation, largely obtained from work on rodents have demonstrated that sickness like behaviors including fever, social exploration and others, are abrogated in vagotomized animals injected systemically with infectious/inflammatory agents such as lipopolysaccharide (LPS). Our own studies on fever have provided evidence that a humoral factor, probably the cytokine interleukin (IL)-6, contributes significantly to transmitting signals to the brain. This is supported by the fact that circulating IL-6 concentration increase dramatically following LPS administration and that this increase correlates well with the development of the febrile response in rats. More convincingly our recent studies using a neutralizing antiserum raised against rat IL-6, have shown that systemic administration of this antiserum totally abolished LPS induced fever in rats. These results support strongly a role for IL-6 as a mediator of peripheral signals to the brain during infection or inflammation induced fever.

Brain Endothelial Cells Are the Primary Sites for Cytokine Action and Prostaglandin Synthesis

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Many of the cytokine actions on the CNS are mediated through production of prostaglandin E_2 (PGE_2) in the brain. To understand this mechanism, we have been studying expression of enzymes responsible for PGE_2 biosynthesis in the rat brain, and its relevance to fever after challenged with cytokines or lipopolysaccharide (LPS). The results were summarized as follows. (1) Cyclooxygenase-2 (COX-2), an inducible-type enzyme converting arachidonic acid to PGH_2, was induced in brain endothelial cells in response to cytokines and LPS. (2) COX-2 expression was correlated with fever in terms of timing and magnitude. (3) Inhibition of COX-2 activity suppressed fever. (4) Microsomal-type PGE synthase (mPGES), another key enzyme that converts PGH_2 to PGE_2, was also induced in brain endothelial cells after LPS challenge. (5) mPGES was colocalized with COX-2 in the perinuclear region of the endothelial cells. (6) Inhibition of COX-2 activity suppressed PGE2 level in the brain. (7) Endothelial cells are the only cell group that expresses both COX-2 and mPGES in the brain. (8) Cytokine receptors are expressed in brain endothelial cells. These results clearly indicate that brain endothelial cells are the primary sites for cytokine-induced PGE2 production, and, hence, play a pivotal role in the immune-brain signaling.

Fever Induction by Endotoxin: Are Cytokines, Prostaglandins, or Other Molecules the Primary Signals?

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The prevailing model of the pathogenesis of fever suggests that exogenous pyrogens, e.g., bacterial endotoxic lipopolysaccharides (LPS), stimulate mononuclear phagocytes (MP) to produce endogenous pyrogenic cytokines, e.g., interleukin (IL)-1, IL-6, and others, that are released into the circulation, eventually reaching the preoptic-anterior hypothalamic region (the primary site of the body's temperature controller in the brain) and stimulating its production of prostaglandin (PG) E2, the putative proximal mediator of the elicited febrile response. Still controversial are the identity of the 'essential' pyrogenic cytokine, the mechanism by which its peripheral signal is transduced into central nervous signals, and the cell source and its exact location in the hypothalamus and the target receptor subtype of the fever-mediating PGE2. Recently, a new quandary has developed, based on the recognition that the febrile response to an intravenous (iv) injection of LPS develops before these cytokines, which are not constitutively expressed in MP, are detectable in blood or tissue. Similarly, the PGE_2 that mediates the febrile response in the brain is evidently produced only on stimulation via a reaction catalyzed by a specific, inducible enzyme, cyclooxygenase (COX)-2. The synthesis of these factors requires more time (60–90 min) than the observed latency of fever onset (~ 10 min, e.g., in guinea pigs). To be valid, therefore, the premise that a peripheral cytokine and central PGE_2, in that order, provide the triggering signals for fever production would require, 1) that the cytokine in question be constitutively expressed, i.e., its cell source could not be MP, and 2) that the brain PGE_2 be derived either via a COX-2-independent synthetic pathway or originate from a source that expresses COX-2 constitutively. Alternatively, the initiating peripheral agent may not be a cytokine. We have investigated whether the complement (C) cascade, which is activated within seconds by iv LPS, may provide the fever-triggering stimulus, and have evidence that C5 is implicated in the febrile response. Mast cells, which express IL-1β and store it preformed within their granules, are non-phagocytic targets of C5. C5 also rapidly stimulates COX-1-mediated PGE_2 production by unstimulated MP. In the brain, two phases of PGE_2 release may occur, possibly arising from different cell sources and mediated, successively, by constitutive and induced COX-2. This presentation will briefly review the current concepts of pyrogen sensing and signaling with respect to the temporal discrepancies between fever onset and the elaboration of putative mediators after iv LPS, and present new data concerning the involvement of C and COX-2 in the pathogenesis of endotoxic fever.

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Cytokines and Behavior: Effects on Learning, Memory, Depression, Sleep, and Appetite

Differential Time-Dependence Sensitization Effects Elicited by Tumor Necrosis Factor-α: HPA Activation, Sickness Behavior and Brain Monoamine Activity

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Stressor and cytokine challenges provoke immediate as well as protracted effects on behavior, hypothalamo-pituitary adrenal (HPA) activity and brain monoamine utilization. Much like interleukin-1β (Tilders and Schmidt, 1998), a single ip injection of tumor necrosis factor-α (TNF-α) induced a sensitization of HPA activity, such that reexposure to the cytokine 2–4 weeks after pretreatment resulted in a pronounced elevation of plasma ACTH and corticosterone levels (Hayley et al., 1999, 2000). Systemic TNF-α also provoked a delayed increase of arginine vasopressin and corticotropin releasing hormone immunoreactivity within the median eminence, which became evident 1–2 weeks following administration of the cytokine but declined after 4 weeks. Thus, variations of these ACTH inducing neuropeptides alone may not fully account for the hormonal sensitization observed after the later interval (4 weeks). Central administration of TNF-α had less profound effects on HPA functioning than did peripheral administration. Paralleling the HPA changes, indices of sickness behavior (reduced feeding, locomotion and social interaction) were also augmented upon reexposure to TNF-α after lengthy time intervals following initial ip administration of the cytokine. In contrast, mice reexposed to TNF-α 1 day following pretreatment with the cytokine exhibited a sensitization of norepinephrine and serotonin utilization within the medial prefrontal cortex and central amygdala. Likewise, pretreatment with the bacterial endotoxin, lipopolysaccharide, greatly increased serotonin utilization within the central amygdala upon administration of TNF-α 1 day following endotoxin challenge. This cross-sensitization was diminished by peripheral depletion of macrophages, suggesting endotoxin primed macrophages may influence brain monoamines upon subsequent TNF-α treatment. It is proposed that TNF-α engenders a remarkable degree of central plasticity which is likely mediated by extensive 'cross-talk' between peripheral (activated immune cells, vagal afferents) and neuroendocrine/neurotransmitter circuits. These data may be important for disorders involving protracted behavioral or neurological symptoms (depression, multiple sclerosis).

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Analysis of the Mechanisms Underlying the Inhibitory Effect of IL-1β on Long-Term Potentiation in Rat Dentate Gyrus

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It has been shown by several groups that IL-1β inhibits long-term potentiation (LTP) in CA1, CA3 and dentate gyrus in vitro and we have recently shown that intracerebroventricular injection also inhibits LTP in dentate gyrus of urethane anaesthetized rats. Our evidence suggests that this effect is mediated by phosphorylation of the stress-activated kinases, p38 and JNK, which in turn inhibit glutamate release. These findings are supported by our recent observations which indicate that lipopolysaccharide (LPS), which increases IL-1β concentration in hippocampus by activating ICE, also increases activation of p38 and JNK, decreases glutamate release and inhibits LTP. Evidence will be presented which indicates that IL-1β and LPS treatment, perhaps by activating p38 and JNK, lead to changes in entorhinal cortex (the cell bodies of perforant path-granule cell synapses) and hippocampus, which are consistent with apoptotic cell death.

Brain Interleukin-1 and Memory Formation

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A variety of cognitive impairments have been reported to occur during infection, autoimmune disease, and cytokine pharmacotherapy. These are all states during which peripheral and central cytokine levels are above normal. These phenomena, together with the finding that elevations in brain levels of interleukin-1β (IL-1β) interfere with long-term potentiation in the hippocampus, led us to explore the effects of peripheral and central IL-1β on learning and memory performance. Both the central application of IL-1β and a number of manipulations that induced IL-1β in brain (peripheral injection of LPS, zymosan, and IL-1-β, central injection of gp120, social isolation) interfered with memory when given after learning trials, and the interference with memory was prevented by intracerebroventricular pretreatment with IL-1 receptor antagonist. Furthermore, this interference with memory was specific to learning tasks that depend on the functioning of the hippocampus and hippocampal long-term potentiation. The finding that none of the above manipulations alter memory on tasks that do not require hippocampal activity eliminates explanations based on interference with only performance. Finally, data will be presented that indicate a role for tumor necrosis factor-α in these phenomena.

Cytokines in Physiological Sleep Regulation

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Many cytokines enhance sleep; the list includes IL1α, IL1β, IL2, IL6, IL15, IL18, EGF, acidic FGF, NGF, GM-CSF, BDNF, GDNF, IFNα, IFNγ, TNFα, and TNFβ. In contrast, anti-inflammatory cytokines IL4, IL10, IL13, TGFβ-1 and IGF-1 inhibit sleep. The size of the list, the fact that many, if not all, of these substances can be made in the CNS, that their receptors are found in the CNS and that many alter firing rates of hypothalamic neurons collectively suggest that a cytokine cascade operates to regulate sleep. However, only IL-1β and TNFα have been studied extensively for their involvement in physiological sleep regulation. Central or systemic injection of exogenous IL1β or TNFα enhances non-rapid eye movement sleep if low doses are given; high doses inhibit sleep. Inhibition of either IL1β or TNFα using antibodies, soluble receptors or antagonists inhibits spontaneous sleep. These inhibitors also inhibit the expected sleep rebound after sleep deprivation. Substances that enhance the production of IL1β or TNFα, e.g., bacterial and viral products, enhance sleep after low doses. Substances which inhibit production of IL1β or TNFα inhibit spontaneous sleep, e.g., CRH. IL1β and TNFα are constitutively expressed in brain and in rats there is a diurnal variation in their mRNA and protein levels with highest concentrations correlating with highest sleep propensity. Sleep deprivation enhances brain levels of IL1β and TNFα mRNA. The IL1 Type 1 and TNF 55 kD receptors are involved in sleep regulation; mice lacking these receptors sleep less than controls. Downstream events in the cascade of events responsible for IL1 and TNF-induced sleep includes NFκB, NO, PGD_2 and adenosine. Many of these substances, including IL1α and TNFα, are also involved in host defense responses and in synaptic plasticity; we hypothesize that sleep plays a functional role in both these processes.

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Cytokine-Mediated Behavioral, Emotional and Cognitive Disturbances in Rodents and Humans

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To investigate the immune-neural mechanisms that mediate sickness behavior we examined in rodents and humans the behavioral effects of various immune challenges and the role of pro-inflammatory cytokines in mediating these effects. We used several animal models of immune activation within the brain, including acute conditions, such as intracerebral administration of *Mycoplasma fermentans* or HIV gp120, and chronic conditions, such as EAE. For example, in the acute phase of EAE, mice display a marked suppression of food and sucrose solution intake, as well as decreased body weight, and social exploration. The expression of pro-inflammatory cytokines within the brain was elevated concurrently with the onset of the behavioral changes, preceding the onset of the first neurological symptoms. Furthermore, a decrease in IL-1 expression was associated with behavioral (but not neurological) recovery. Dexamethasone, a steroid anti-inflammatory drug, but not pentoxifylline, a TNF-α synthesis inhibitor, attenuated EAE-induced sickness behavior, suggesting that pro-inflammatory, but not specifically TNF-α, are essential in mediating the EAE-associated behavioral syndrome. In studies with humans, we used a double-blind prospective design to investigate the psychological consequences of immune activation in several experimental models. For example, we found that compared to control (saline) levels, injection of LPS in healthy volunteers resulted in elevated serum levels of proinflammatory cytokines and cortisol, as well as mild fever and anorexia. LPS also induced a significant elevation of negative emotions, particularly depression and anxiety, as well as cognitive alterations, particularly impaired learning and memory. Furthermore, a high correlation was found between LPS-induced TNFα and IL-6 secretion and the psychological disturbances. In conclusion, in both animals and humans, stimulation of the primary host defense is associated with cytokine-mediated behavioral, emotional and memory disturbances. Hence, cytokines represent a novel target for neuropsychopharmacological research.

What Is the Evidence for a Role of Cytokines in Depression?

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To investigate the possible involvement of cytokines in depression, two series of studies have been carried out. At the experimental level, chronic but not acute treatment with the atypical antidepressant drug tianeptine attenuates the behavioral and neuroendocrine effects of IL-1 and LPS. These effects are obtained only when IL-1 and LPS are injected ip but not icv, which indicates that tianeptine does not alter the sensitivity of brain targets to cytokines. Preliminary results indicate that these effects are accompanied by a shift in the balance of IL-1β/IL-10 expression in the brain in response to LPS, suggesting that tianeptine might alter the balance between pro- and anti-inflammatory cytokines. At the clinical level, studies in cancer patients treated with IL-2 and IFN-α show a differential effect of these cytokines on mood and cognition. In particular, changes in mood occur earlier in response to IL-2 than to IFN-α. Furthermore, patients with relatively higher scores of depression at the initiation of treatment are more likely to develop depressive episodes than patients with lower scores, suggesting the existence of an individual vulnerability to the depressive effects of cytokines. These results are important since they allow to identify patients at risk to treat them accordingly.

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Poster Abstracts

Microglial Activation and Interleukin-1β Expression in the Preoptic Area/Anterior Hypothalamus following Middle Cerebral Artery Occlusion

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The intraluminal filament technique that is commonly used for middle cerebral artery occlusion in animal models of stroke is known to produce spontaneous hyperthermia. This hyperthermia increases the infarct volume, especially if it occurs during the first six hours after the onset of the ischemia, and is presumed to be caused by ischemic damage in the heat regulatory system of the hypothalamus. Microglial activation and IL-1β expression were examined in the rat hypothalamic heat regulatory system, the preoptic area, following middle cerebral artery occlusion. Microglial cells and the expression of IL-1β were studied with immunohistochemistry during the first six hours after the onset of the occlusion. Although only the lateral preoptic area and the magnocellular preoptic nucleus were involved in the infarct two days after the middle cerebral artery occlusion, microglial activation and IL-1β-containing microglial cells were detected in the lateral preoptic area as well as the anterior medial nucleus, the anteroventral nucleus, and the medial preoptic area. Activated and IL-1β-containing microglial cells were found in both the ipsi- and contralateral medial preoptic area. It is proposed that the activation of microglial cells and their production of cytokines play a role in the generation of the spontaneous hyperthermia following middle cerebral artery occlusion.

Plasma and Cerebrospinal Fluid Interleukin-6 Concentrations in Combat Veterans with Posttraumatic Stress Disorder

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Background: In an attempt to replicate a preliminary report of increased plasma interleukin-6 (IL-6) in patients with posttraumatic stress disorder (PTSD), and to extend examination of IL-6 in this population into the CNS, we determined cerebrospinal fluid (CSF) and plasma concentrations of this cytokine. We also assessed the relations between IL-6 levels and hypothalamic-pituitary-adrenal and adrenergic activities. **Methods:** CSF was withdrawn via a subarachnoid catheter over 6 h from 11 combat veterans with PTSD and age- and sex-matched controls. Blood was withdrawn concurrently. Plasma IL-6 concentrations were quantified on all subjects, and CSF IL-6 concentrations were determined on a subgroup of both cohorts. IL-6 concentrations were correlated with CSF corticotropin-releasing hormone (CRH), ACTH, cortisol and CSF and plasma norepinephrine levels. **Results:** Mean and median CSF IL-6 concentrations were higher in PTSD than in controls (mean = 24.00 vs 14.56, $p = 0.05$; median = 26.71 vs 14.25, $p < 0.03$). Plasma IL-6 concentrations were not different between the two groups. Plasma IL-6 and norepinephrine were positively correlated in the PTSD group ($r = +0.74$, $p < 0.04$), but not in normals ($n = -0.55$, n.s.). **Conclusions:** Daytime basal plasma IL-6 concentrations are not increased in PTSD, although plasma IL-6 appears to be more tightly associated with noradrenergic output in these patients than in normals; yet, CSF IL-6 is increased in the former. Both findings might be explained by the low cortisol secretion previously reported in PTSD as a result of lowered glucocorticoid suppression of IL-6 secretion. High levels of CSF IL-6 may reflect or be associated with neurodegeneration.

Dorsal Hippocampal Injection of Interleukin-1β (IL-1β) Impairs Memory Consolidation

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The pro-inflammatory cytokine IL-1β injected intracerebroventricularly (ICV) disrupts memory consolidation in a hippocampal-dependent contextual fear conditioning paradigm. We now report that consolidation processes in the dorsal hippocampus are disrupted by IL-1β. When normal Sprague Dawley rats are placed into the context and immediately shocked and removed, they later display very little contextual fear, as they did not have sufficient exposure to form a memory representation of the context. Preexposure to the context (6 times in 20 min.) the day before conditioning, however, enhances contextual fear in rats shocked (2 s, 0.65 mA) immediately, indicating that rats learn about the context during preexposure. Here we show that this preexposure facilitation effect is markedly attenuated in rats that received post-training, bilateral intra-dorsal hippocampus injections of IL-1β (0.5 ng in 0.5 ml) but not in vehicle-injected rats ($F = 10.36$, $p < 0.01$). These data suggest that the dorsal hippocampus is one site of action for IL-1β -mediated disruption of memory consolidation in this contextual fear conditioning paradigm.

Cytokines and Sickness Behavior Are Attenuated by Short Day Lengths in Siberian Hamsters

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Infected animals exhibit a stereotypical repertoire of so-called 'sickness behaviors' that include anorexia, fever, and lethargy. Fever

and anorexia are energetically costly, however, and ill individuals may suspend or override these sickness behaviors in response to specific intrinsic energy signals. Although adaptive during most infections, anorexia and fever are potentially problematic during winter and other times of energy shortage. We hypothesized that these energy-demanding aspects of sickness behaviors may not occur in infected Siberian hamsters (*Phodopus sungorus*) housed in short days, because winter-simulated day lengths evoke dramatic body mass loss to conserve energy. To test these hypotheses, male and female hamsters were housed for 8 weeks in either LD 10:14 or LD 14:10 photoperiods. All animals were assessed for anorexia, fever, and lethargy following an acute challenge with lipopolysaccharide (LPS). Short-day Siberian hamsters significantly limit anorexia in conjunction with body mass loss, and the neuroendocrine mediators IL-1, IL-6, leptin and cortisol are decreased in short days as well. These data suggest that seasonal alterations in body mass and energy availability may modulate the expression of sickness behaviors in Siberian hamsters, and that sickness behaviors may be mediated by cytokines in this species.

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Effects of Interferon α Single Administration on Growth Hormone mRNA Expression in Rat Anterior Pituitary

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Background: Interferon α (IFNα) is a cytokine with pleiotropic effects and influences secretion of certain cytokines and hormones. From the other hand, Growth Hormone (GH) secreted from the pituitary has physiological effects on various target tissues. The question is how the IFNα administered various diseases influences GH secretion . The aim of the present study was to measure the cellular expression of GH mRNA by in situ hybridization. **Materials and Methods:** The 45 intact male Wistar rats (7–8 weeks, 250 mg.c.w.) were considered into experiment. Rats were administered intraperitoneal injection of human IFNα (examined groups) or saline (control group). Rat pituitaries were taken 2 and 4 h after IFNα/saline administration and kept frozen until in situ hybridization histochemistry. 12-μm sections were taken through the pituitary. 46-base ^{35}S-labelled oligonucleotide probes complementary to part of the exonic mRNA sequences coding for GH mRNA were used. All control and experimental sections. Results are presented as the mean percentage change from control ± SE. **Results:** IFNα acute administration slightly (not significantly) increases GH mRNA expression in the anterior pituitary in 4-hour group in comparison to the control group, and there was observed no difference between control group and 2-hour rats.

Cytokine and Neurotrophin mRNA Expression by Mixed Cortical Glia: Ontogeny and Differential Responses to Hypoxia-Reoxygenation Injury

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Primary mixed cortical glia cultures were used to examine elevations in mRNA levels for neurotrophins and pro-inflammatory cytokines following moderate hypoxia-reoxygenation at 12, 21, and 28 days in vitro (DIV). In normoxic controls, mRNA levels for βNGF, BDNF, GDNF, CNTF, and NT4 were highest at 12 DIV while, IL-1α, IL-1β, IL-6, and TNFα peaked at 21 DIV. At 21 DIV, 6 h of hypoxia produced significant elevations in mRNA levels for both cytokines and neurotrophins which was inhibited by co-exposure to 5 nM dexamethasone. In older cultures (28 DIV), no elevation was seen with hypoxia however, following 3 h of reoxygenation, mRNA levels were slightly elevated for IL-1α, IL-1β, and TNFα, 500% for IL-6 mRNA levels, and 200% for and βNGF, BDNF, and GDNF. Co-administration of dexamethasone inhibited the IL-1α, IL-1β, and TNFα responses and exacerbated the IL-6 response. No effect was seen on the hypoxia-reoxygenation induced elevation in βNGF, BDNF, and GDNF mRNA levels. At both 21 and 28 DIV, levels returned to normoxic baseline by 24 h reoxygenation. From these data we conclude that hypoxic insult, without loss of other substrates (ischemia), is sufficient stimulus for pro-inflammatory and neurotrophin responses by mixed glia dependent upon age of culture.

Interleukin-6 Is Non-Pyrogenic in Mice, but Augments Interleukin-1β-Induced Fever

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To clarify possible cytokine interaction in fever genesis, interleukin (IL)-1β (2.5 ng/mouse) and IL-6 (1 μg mouse) were injected alone or in combination in mice via the intracerebroventricular (i.c.v.) route, and their actions on fever were examined. IL-6 alone did not evoke fever in wild-type mice and those with disrupted IL-1β converting enzyme (ICE) gene, which is essential for the production of mature IL-1β. IL-1β alone evoked fever in both types of mice although fever was less intense in ICE gene disrupted mice and in wild-type mice pretreated with ICE inhibitor, indicating exogenous IL-1β induced endogenous production of IL-1β which augmented the fever. Combined injection of IL-1β with IL-6 resulted in more pronounced fever than the single injection of IL-1β in both types of mice and wild-type mice pretreated with the ICE inhibitor. The augmentation by IL-6 was more pronounced in mice with disrupted ICE gene or those pretreated with the ICE inhibitor indicating endogenous production of IL-1β veiled the action of IL-6. The fever was sup-

pressed with an inhibitor of cyclooxygenase-2, an enzyme responsible for prostaglandin biosynthesis. These results suggest that IL-1β and IL-6 synergistically evoke fever in mice.

Indomethacin (Cyclooxygenase Inhibitor) Suppresses the Interleukin-1β Concentrations in Human Cerebral Spinal Fluid after Subarachnoid Hemorrhage

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Overexpression of interleukin-1β (IL-1β) after subarachnoid hemorrhage (SAH) contributes to secondary brain damage and vasospasms. Indomethacin, a non-selective cyclooxygenase (COX) inhibitor, has several effects including of anti-cytokines. We developed a novel pharmacological brain cooling (PBC) by indomethacin. We also measured human CSF levels of IL-1β and compared with normal treated patients (non-PBC group) in this study. **Patients and Methods:** From 1997 to 2000, we analyzed 35 patients with subarachnoid hemorrhage (SAH) admitted to the Department of Neurosurgery at Showa University Hospital. Brain core temperature (BT) was measured directly with a ventriclostomy catheter. Patients were cooled (37.5°C > BT) by using trans-rectal indomethacin (100 mg) and were maintained at this temperature by 6 mg/kg/day dose of indomethacin for 14 days. We also measured CSF levels of interleukin-1β at Day 1, 2, 4, 7 and 14 after SAH (ELISA). **Results and Conclusions:** BT was controlled in target temperature (37.5°C) within 24 h. Complicating rate of symptomatic vasospasms was lower than non-PBC group. CSF levels of IL-1β after SAH were suppressed in PBC group. PBC also significantly decreased the complication of vasospasms. An initial experience of PBC in SAH appears to yield favorable outcomes and acceptably low rate of systemic complications and vasospasms through suppression of IL-1β.

Cyclooxygenase Isozyme Involvement in Interleukin-1-Induced Hypophagia in Mice

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Interleukin-1 (IL-1) administration induces hypophagia in mice, mimicking the sickness behavior induced by endotoxin (LPS) and viral infections. Extensive pharmacological studies have failed to identify the precise neural mechanisms involved (Swiergiel et al., Pharmacol. Biochem. Behav. 63: 629–637, 1999; Swiergiel and Dunn, Pharmacol. Biochem. Behav. 65: 531–537, 2000). However, cyclooxygenase (COX) inhibitors largely prevent the response to IL-1, but are less effective against LPS and influenza virus infection (Swiergiel et al., Pharmacol. Biochem. Behav. 57: 389–396, 1997). The location of the relevant COX enzyme is unknown, but could be associated with endothelial cells. Our studies with selective inhibitors of COX1 and COX2 have been inconclusive. The selective COX1 inhibitor, piroxicam more or less prevented the hypophagic response to IL-1, and COX2-selective inhibitors such as nimesulide and NS-398 antagonized the response only at higher doses (Dunn and Swiergiel, Brain Behav. Immun. 14: 141–152, 2000). Nevertheless, the highly selective COX2 inhibitor, celecoxib, largely prevented the reduced intake of sweetened milk normally observed following ip injection of 100 ng mIL-1β at doses of 1, 3 or 10 mg/kg. We have now studied the hypophagic effects of IL-1 in mice deficient in COX1 and COX2. COX1-knockout mice exhibited a normal reduction in milk intake following mIL-1β. The results with COX2-knockout mice were quite variable, but some response to IL-1 was observed in some mice. Taken together, these results suggest that COX2 may be the major isozyme involved in the hypophagic effects of IL-1. However, the time course of the IL-1-induced hypophagia is much shorter than the reported induction of COX2 activity in cerebral endothelial cells.

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C-fos Expression in the Brain in the Systemic GvH Reaction

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Purpose: It is hypothesized that the brain receives information about peripheral immune reactions and feedbacks them through autonomic and endocrine systems. Although it is reported that the peripheral injections of LPS and IL-1β induced c-fos expressions in the specific brain areas related to autonomic and neuroendocrine functions, it is not clear whether the same phenomenon can be observed during specific immune responses. To explore this possibility, we have studied c-fos expression in the brain during GvH response. **Methods:** (WKY × PVG)F1 rats were injected i.v. 5×10^8 spleen cells of PVG or F1 rats and studied c-fos expression by immunohistochemistry 1, 4, 7 and 10 days after spleen cells injection. **Results and Discussion:** We have observed that on day 4 GvH animals showed stronger c-fos expressions in the brain areas related to olfactory and visual functions compared with control animals. In this GvH model, there were no c-fos expression in the brain regions (NTS, OVLT, PVN, LC) reported in LPS and IL-1β injections. C-fos IR cells could possibly be neurons judging from distribution patterns and cell concentrations. However the possibility of other cell types cannot be excluded. Our results suggest that immune cells can convey signals to the brain via stimulation of olfactory and optic neural pathways.

The Inflammatory Cytokines IL-1, IL-6 and TNF Mediate Neuroprotection to NMDA, β-Amyloid or Paraquat through Different Pathways

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IL-1, IL-6 and TNF are expressed constitutively in some cells of the brain and are elevated in brains of animals challenged peripherally with an inflammatory substance. The role of these cytokines in the central nervous system during events that challenge neuronal viability is under investigation. Cultured cortical neurons challenged with neurotoxins undergo cellular processes related to necrosis or apoptosis that eventually result in neuronal death. Pretreatment of primary cortical neuronal cultures with either IL-1 (α or β), IL-6, or TNFα protects neurons to a subsequent challenge with either n-methyl-d-aspartic acid (NMDA), β-amyloid peptide, or paraquat. We have determined that the requirements for cytokine-induced neuroprotection vary for each cytokine. For TNF, autocrine activity, TNFRI expression, and ceramide-induction are required. For IL-1 and IL-6, NGF release and IL-1RI expression are necessary to achieve neuroprotection. Further, we have inferred differing mechanisms through which cytokines impart neuroprotection by examining the effects of both nicotine and alcohol on cytokine efficacy. Both nicotine and alcohol inhibit TNF-induced neuroprotection, but they have no effect on either IL-1 or IL-6-mediated processes. These results demonstrate the ability of these naturally-occurring CNS-cytokines to function as neuroprotectants to a variety of neurotoxic agents, and that the cellular mechanisms involved in accomplishing neuronal survival are diverse.

The Role of Vagus Nerve in Interleukin-1β-Induced Fever Is Dependent on Dose

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It has been suggested that pro-inflammatory cytokines communicate to the brain via a neural pathway involving activation of vagal afferents by interleukin-1β (IL-1β), in addition to blood-borne routes. In support of this possibility, subdiaphragmatic vagotomy has been reported to block IL-1β induced brain-mediated responses such as fever. However, vagotomy has also been reported to be ineffective. Neural signaling would be expected to be especially important at low doses of cytokine, when local actions could occur with only very small quantities of cytokine becoming systemic. In this study, we examined core body temperature after intraperitoneal injections of three doses of recombinant human IL-1β (rhIL-1β: 0.1, 0.5, 1.0 mg/kg). Subdiaphragmatic vagotomy completely blocked the fever produced by 0.1 mg/kg, partially blocked the fever produced by 0.5 mg/kg, and had no effect on the fever that followed 1.0 mg/kg rhIL-1β. In addition, blood levels of rhIL-1β did not become greater than normal basal levels of endogenous rat IL-1β until the 0.5 mg/kg dose, nor was IL-1β induced in the pituitary until this dose. These results suggest that low does of intraperitoneal IL-1β induce fever via a vagal route and that dose may well account for some of the discrepancies in the literature.

Characterization and Visualization of ^{125}I-Labeled Stromal Cell-Derived Factor-1α Binding to CXCR4 Receptors in Rat Brain and Human Neuroblastoma Cells

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Stromal cell-derived factor-1α (SDF-1α) binds to the seven-transmembrane G-protein-coupled CXCR4 receptor and modulates cell migration, differentiation, and proliferation. CXCR4 has been reported to be expressed in various tissues including brain. Moreover CXCR4 has recently been shown to be one of the coreceptors for HIV-1 infection which could be implicated in HIV encephalitis. In the present study, the binding properties and autoradiographic distribution of [^{125}I]SDF-1α binding to CXCR4 were characterized in the adult rat brain. SDF-1α binding and CXCR4 coupling system were also studied in a human neuroblastoma cell line SK-N-SH. The binding of [^{125}I]SDF-1α on rat brain sections was specific, time-dependent and reversible. The highest densities of [^{125}I]SDF-1α binding sites were detected in the choroid plexus of the lateral and the dorsal third ventricle. Lower densities of [^{125}I]SDF-1α binding sites were observed in various brain regions including cerebral cortex, anterior olfactory nuclei, hippocampal formation, thalamic nuclei, blood vessels and pituitary gland. In the choroid plexus, the IC_{50} and K_d of [^{125}I]SDF-1α binding were respectively 0.6 n*M* and 0.36 n*M*. Similar IC_{50} values were obtained in other brain structures. Immunohistochemical studies by an anti-CXCR4 antibody confirmed the presence of CXCR4 receptors in the rat brain sections. In SK-N-SH cells, [^{125}I]SDF-1α bound to CXCR4 with a K_d of 5.0 n*M* and a maximal binding capacity of 460 fmol/mg of protein. SDF-1α induced a rapid and transient intracellular calcium increase in SK-N-SH cells. These findings suggest that CXCR4 is expressed in some brain structures and may have a regulatory role in the nervous system.

Decreased Neuronal Cell Death after Global Ischemia in Mice Lacking TNF-α

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TNF-α, one of the pro-inflammatory cytokines, is increased after brain ischemia and it may cause neuronal cell death induced by brain

administered 60 min prior to training, with experimental chicks responding to the task only 73% as well as controls. Retention tests revealed deficits in memory processing were evident by 20 min post-training. These results demonstrate an inhibitory effect of LPS on memory processing at the transition point from short-term memory to intermediate-term memory.

Lack of Effect of Lipolysaccharide and Interleukin-1β on the Expression of a Conditioned Place Preference in Rats

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Recently, immune system activation has been shown to induce anhedonia, or decreased interest in pleasurable stimuli. Studies have predominately assessed the effect of cytokines or lipolysaccharide (LPS) on behavior maintained by primary reinforcers. These stimuli, such as palatable solutions, are effective in reinforcing behavior without prior training. We were interested in replicating the anhedonic effects of immune system activation on primary reinforcers, in addition to assessing the effects of sickness on behavior maintained by a secondary reinforcer, a stimulus paired with a primary reinforcer. Using a conditioned place preference procedure, we assessed the effects of interleukin-1β (IL-1β) and LPS on sucrose intake (primary reinforcer) and preference for a sucrose-paired environment (secondary reinforcer). Animals were conditioned to associate sucrose with a distinctive environment in the place conditioning apparatus. Once conditioning had occurred, the effects of LPS or IL-1β on sucrose consumption and the sucrose-induced place preference were assessed. Both IL-1β and LPS significantly decreased sucrose intake but had no effect on the expression of a sucrose-induced place preference. These findings suggest a differential effect of immune system activation on hedonic behaviors maintained by primary and secondary reinforcers.

TNF and TNF Receptors Expression in Trimethyltin Induced Hippocampal Injury

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Trimethyltin (TMT) is an organotypic neurotoxicant which produces a specific pattern of neuronal degeneration in the hippocampus. TMT induces a specific neuronal loss of dentate granule cells, microglia activation, and astrocyte hypertrophy. Previous studies in our laboratory have shown that these morphological changes are associated with an increase of mRNA for tumor necrosis factor (TNF) from 12 to 72 h. We examined the mRNA levels of TNF receptors over time, by ribonuclease protection assay, in hippocampus of 21-day-old CD1 male mice treated with an acute dose of TMT (2.0 mg/kg, ip). An increase of the TNF receptor (TNFR) I was observed, starting at 24 h and reaching a peak at 48–72 h. There was also an upregulation of TNFRII mRNA with a peak at 72 h. These results suggest that in addition to the TNF modulation, the regulation of its receptors may also play a role in the hippocampal lesion. Studies of mRNA expression of TNF and its receptors are currently underway using laser capture microdissection and real time PCR to compare the region with neuronal damage (dentate) to a spared region (CA).

Trimethyltin-Induced Hippocampal Neurodegeneration Elevates Cyclin and Cytokine mRNA Levels

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An acute administration of the hippocampal toxicant, trimethyltin (TMT; 2 mg/kg ip), to 21-day-old CD-1 mice produced, within 24 h, a specific pattern of neuronal necrosis in dentate granule cells with accompanying astrogliosis and initiation of a cytokine response. While microglia shows an early response to injury as indicated by an elevation in mRNA levels for TNFα and IL-1α at 24 h, their morphological progression to an activated phagocytic phenotype does not occur until 72 h post-TMT. This activation is accompanied by a dramatic (10-fold) elevation in mRNA levels for cyclin A2 and cyclin B1. Western blot analysis of hippocampal tissue at 72 h showed a slight increase of cyclin B with no elevation in cyclin A. Immunohistochemistry demonstrated cellular localization of cyclin A to reactive microglia in the pyramidal cell layer and activated glia in the dentate and cyclin B in satellite glia in the dentate. The lack of either PCNA immunostaining or BrdU labeling in cyclin positive cells suggest that the up-regulation of cell cycle genes may be associated with cellular processes other than proliferation and may contribute to the differentiation of microglia to a phagocytic phenotype.

The Effect of Cardiac Arrest on the Blood-Testis Barrier to Albumin, TNF-α, PACAP, Sucrose and Verapamil in the Mouse

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Impotence commonly occurs after events such as acute myocardial infarction, coronary bypass, head trauma, and cerebral bleeding, including subarachnoid hemorrhage. We hypothesize that the hypoxia accompanying these events could damage the blood-testis barrier (BTB) and it may cause testicular dysfunction, a possible cause of impotence. We examined the effect of cardiac arrest in mice on testis weight and various aspects of BTB function. Testis weight was

decreased about 24% by 12 h after cardiac arrest but had recovered fully by day 7. The testis/serum ratio for albumin was increased 12 h after arrest showing a disruption in the vascular BTB with recovery by 24 h. The testis/serum ratio for sucrose was not consistently elevated, showing that the Sertoli cell BTB remained intact. The testis/serum ratio for verapamil was increased on day 3 of cardiac arrest suggesting impaired function of the BTB's p-glycoprotein efflux transporter. Transporters for pituitary adenylate cyclase activating polypeptide (PACAP) and tumor necrosis factor-α (TNF-α) were not affected by cardiac arrest. These results indicate that cardiac arrest affects testis weight and some aspects of BTB function. Such changes might have long term effects on testicular function that could lead to impotence.

Reduction of Infarct Volume after Transient Cerebral Ischemia in Mice Deficient in IL-1

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Expression of IL-1 is reported to upregulate after brain injury and the infarct volume was decreased after inhibiting its action. The role of neurodegeneration in IL-1 is shown, but there has been no direct evidence of its action and mechanism. This study was designed to identify the difference of infarction after ischemia between IL-1α/β gene deficient (IL-1 KO) and the wild-type (WT) mice and to estimate the neurodegenerative mechanism of IL-1 during ischemia. Middle cerebral artery in IL-1 KO and WT mice was occluded for 1 h and reperfused up to 96 h with intrasutural method (tMCAO). The expression of IL-1β mRNA and its immunoreactivity was examined using RT-PCR and immunohistochemistry. The expression of IL-1β mRNA was increased at 6 h in the ipsilateral hemisphere. The immunoreactivity of IL-1β was detected in the ischemic area at 3 h, while intensely expressed in penumbra at 24 h. The injured volume at 48 h after tMCAO, as measured by 2,3,5-triphenyltetrazolium chloride staining, revealed significantly smaller in IL-1 KO than in WT mice, despite similar reductions in regional cerebral blood flow. These results indicate that IL-1 plays an important role in neurodegeneration during ischemic injury.

Relationship of EP_{1-4} Prostaglandin Receptors with Rat Hypothalamic Cell Groups Involved in Lipopolysaccharide Fever Responses

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Prostaglandin E_2 (PGE_2) is assumed to be a principal mediator of cytokine-induced fever such as interleukin-1. Among the four subtypes of E-series prostaglandin (EP) receptors, previous studies suggested the involvement of EP_1 and EP_3 receptors in fever response in rats and mice, respectively. We investigated which subtypes of EP receptors are activated during intravenous (i.v.) lipopolysaccharide (LPS)-induced fever by assessing the coexpression of Fos-like immunoreactivity (Fos-IR) and EP_{1-4} receptor mRNA in the rat hypothalamus. Two hours after the administration of i.v. LPS (5 mg/kg), Fos-IR was observed in the ventromedial preoptic nucleus, the median preoptic nucleus (MnPO), and the paraventricular hypothalamic nucleus. In these nuclei, EP_4 receptor mRNA was strongly expressed and the Fos-IR intensely colocalized with EP_4 receptor mRNA. Strong EP_3 receptor mRNA expression was only seen within the MnPO but Fos-IR showed little coexpression with EP_3 receptor mRNA. EP_2 receptor mRNA was not seen in these nuclei. Although about half of the Fos-immunoreactive neurons also expressed EP_1 receptor mRNA, EP_1 mRNA expression was weak and its distribution was diffuse in the preoptic area. Our findings indicate that neurons expressing EP_4 receptor are activated during LPS-induced fever.

Cytokines Induced by Bacterial Lipoproteins in Primary Cultures of Rhesus Monkey Brain Cells: Their Role as Mediators of Disease in the Central Nervous System

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Brain invasion by *Borrelia burgdorferi*, the agent of Lyme disease, results in an inflammatory and neurodegenerating disorder called neuroborreliosis. The rhesus monkey reproduces the signs of human neuroborreliosis. *B. burgdorferi* has over 150 lipoprotein genes, 10 times the number present in other Gram-negative bacteria. Bacterial lipoproteins have been shown to be potent immunostimulatory agents. We are exploring the hypothesis that mediators elicited by astrocytes and microglia in response to bacterial lipoproteins, constitute the molecular basis for neuroborreliosis. We established primary cultures of rhesus monkey brain cells. Stimulation of either aggregate cultures or pure microglia with recombinant lipidated outer surface

protein A (L-OspA), a model *B. burgdorferi* lipoprotein, resulted in the production of various inflammatory cytokines, e.g. interleukin 6 (IL-6), tumor necrosis factor (TNF-α), IL-12 and IL-1β. Pure astrocytic cultures produced IL-6 and TNF-α in response to L-OspA, but not IL-12 or IL-1β. Cytokines have been implicated in causing apoptosis and gliosis in several CNS disorders. We have now detected apoptosis of rhesus monkey astrocytes when the latter are exposed to L-OspA, TNF-α or IL-1β. The effect of L-OspA, and the cytokines it elicits on neuronal cultures will be studied next.

Is the Arcuate Nucleus Involved in Cytokine-Induced Anorexia?

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Cytokine-mediated anorexia is a component of 'sickness behavior', and presents a significant obstacle in the treatment of chronic illnesses. We hypothesized that the hypothalamic arcuate nucleus (ARH) might be involved in mediating the anorexic effects of a systemic interleukin-1 (IL-1) challenge based on its content of peptidergic populations involved in control of feeding, its expression of type 1 IL-1 receptors, its responsiveness to systemic IL-1, and its ability to be accessed directly by circulating macromolecules. This hypothesis was tested in rats subjected to arcuate lesions produced by neonatal monosodium glutamate injections (4 mg/g, ip). In control animals, recombinant rat IL-1 (~ 3 μg/kg, iv) gave rise to a significant suppression of feeding (to 74.8% of vehicle injected values) during the first 4 h of refeeding after 20 h fast. Unexpectedly, this effect was significantly accentuated in ARH-lesioned animals (to 48.8%, $p < 0.05$). This potentiation was not specific to IL-1-induced anorexia, as the same animals manifested a comparable enhancement of the anorexia induced by fenfluramine (2 mg/kg, ip). In intact rats, IL-1 injection was found to provoke Fos induction preferentially in neuropeptide Y (NPY)-producing neurons in and around the ARH, and only weakly and inconsistently in proopiomelanocortin-expressing cells. These results fail to support ARH mediation of IL-1-induced anorexia. Lesion-induced potentiation of this effect is not specific to the appetite-suppressing effects of cytokines, and may result from destruction of NPY neurons thought to play a role in the stimulatory control of food intake.

Differential Recruitment of Vascular-Associated Cell Types to Interleukin-1 versus Endotoxin

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Induced prostanoid synthesis by cells associated with the cerebral vasculature has been implicated in mediating immune system influences on the CNS, but the cell type(s) involved remain unsettled. To determine whether this might derive from differences in the nature and/or intensity of the stimuli used to model immune insults, immunoreactive cyclooxygenase-2 (COX-2-ir) was examined in brains of rats perfused 2–4 h after intravenous interleukin-1 (IL-1; 2 or 10 μg/kg) or bacterial lipopolysaccharide (LPS; 2 or 100 μg/kg). Dual immunoflourescence methods were used to localize COX-2-ir and perivascular (ED2 antigen) or endothelial (RECA-1) cell markers. Vehicle-treated rats displayed weak Cox-2-ir in the meninges, choroid plexus and larger blood vessels; greater than 90% were ED2-positive. Rats sacrificed 2 h after either dose of IL-1β showed a marked increase in the number of vascular-associated cells displaying COX-2- and ED2-irs. Rats perfused 2–4 h after LPS displayed an even greater number of COX-2-ir profiles which exhibited distinct round or stellate morphologies, corresponding to cells expressing the RECA-1 or ED2 markers, respectively. Similarly, ultrastructural analysis localized COX-2-ir to the perinuclear region of endothelial cells of LPS-, but not IL-1-treated rats. We conclude that a moderate IL-1 challenge is sufficient to elicit COX-2 induction in perivascular cells, while endothelial cells require one or more facets of the complex immune stimulus presented by LPS.

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